bio-protocol Preprint

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Cell culture and transfection

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An abbreviated version of this protocol was published in Science in Nov 2020

Neuropilin-1 is a host factor for SARS-CoV-2 infection

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Detailed protocol

Calu-3, Caco-2 (a kind gift from Dr Darryl Hill), Caco-2 shSCR and shNRP1 (a kind gift from Giuseppe Balistreri), HeLa, HEK293T and Vero E6 cell lines were originally sourced from the American Type Culture Collection. Authentication was from the American Type Culture Collection. We did not independently authenticate the cell lines. Cells were grown in DMEM medium (Sigma-Aldrich) supplemented with 10% (vol/vol) FCS (Sigma-Aldrich) and penicillin/streptomycin (Gibco) with the exception of Calu-3 cells that were grown in Eagle's minimal essential medium (MEM+GlutaMAX; GibcoTM , ThermoFischer) supplemented with 10% FCS 0.1mM non-essential amino acids (NEAA), 1mM sodium pyruvate, 100 IU/ml streptomycin and 100 µg/ml penicillin. Caco-2 cells were maintained in DMEM+GlutaMAX, 10% FCS and 0.1mM NEAA. FuGENE HD (Promega) was used for transient transfection of DNA constructs for infection assays according to the manufacturer's instructions. PPC-1 human primary prostate cancer cells were obtained from Erkki Ruoslahti laboratory at Cancer Research Center, Sanford Burnham-Prebys Medical Discovery Institute. M21 human melanoma cells were obtained from David Cheresh at University of California San Diego. Cos-7 cells were obtained from Urs Greber laboratory. Cells were cultured in DMEM medium containing 100 IU/mL of streptomycin, penicillin, and 10% FBS in 37°C incubator with 5% CO2.

To generate a NRP1-null HeLa cell line, the following guide RNA (gRNA) was cloned into pSpCas9(BB)-2A-Puro (PX459): 5'-GATCGACGTTAGCTCCAACG-3'. gRNA was transfected into HeLa cells using FuGENE HD. 24 hours later, transfected cells were selected with puromycin. Selected cells were trypsinised and diluted to a concentration of 2.5 cells mL-1 in Iscove's modified Dulbecco's medium (Gibco) supplemented with 10% (vol/vol) FBS (Sigma-Aldrich). 200 µL of this suspension was plated into 96-well plates to seed single cell colonies. After three weeks, colonies were expanded and lysed, and knockout was validated by immunoblotting for NRP1.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Simonetti, B. , Cullen, P. and Yamauchi, Y. (2021). Cell culture and transfection. Bio-protocol Preprint. bio-protocol.org/prep1380.
2. Daly, J. L., Simonetti, B., Klein, K., Chen, K., Williamson, M. K., Antón-Plágaro, C., Shoemark, D. K., Simón-Gracia, L., Bauer, M., Hollandi, R., Greber, U. F., Horvath, P., Sessions, R. B., Helenius, A., Hiscox, J. A., Teesalu, T., Matthews, D. A., Davidson, A. D., Collins, B. M., Cullen, P. J. and Yamauchi, Y.(2020). Neuropilin-1 is a host factor for SARS-CoV-2 infection. Science 370(6518). DOI: 10.1126/science.abd3072